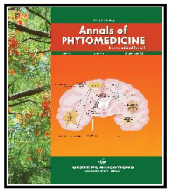
Annals of Phytomedicine 12(1): 538-545, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.1.12

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Antifungal effects of plant essential oils against *Colletotrichum gloeosporioides*, the fungus associated with citrus twig blight

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Article Info

Article history
Received 7 January 2023
Revised 1 March 2023
Accepted 2 March 2023
Published Online 30 June-2023

Keywords
Citrus
Colletotrichum gloeosporioides
Twig blight
Essential oil
Antifungal activity

Abstract

Twig blight is the most common disease of citrus species caused by *Colletotrichum gloeosporioides* in the Vidarbha region of Maharashtra State, India, resulting in canopy reduction and fruit retention in orchards. The poison food method and the volatile phase effect were used in this work to evaluate nine essential oils for their antifungal efficacy against C. *gloeosporioides* at levels of 0.1, 0.5, and 1%. The essential oils studies revealed a broad spectrum of inhibitory activity against *C. gloeosporioides*, even at the lowest dose. The poison food method revealed that the essential oils of citronella, lemongrass, and garlic were more efficient and produced 100% inhibition at the concentrations tested. The promising results of the volatile phase method also show that lemongrass, citronella, palmarosa, and garlic oils all strongly inhibited (100%) the pathogen at the concentrations tested. This demonstrates the volatile nature of these essential oils. The current studies also revealed that the percentage of inhibition was dose-dependent, with higher concentrations resulting in greater inhibition.

1. Introduction

Among commercially grown citrus species, Nagpur mandarin (*Citrus reticulata*-Blanco; Family: Rutaceae) is the most important citrus fruit grown in India. The fruits are commonly used in the daily diet of Indians for squash, syrups and table purposes. One mandarin fruit contains 26% of the daily requirement of vitamin 'C'. Mandarin is rich in vitamin C. Due to their oxidizing properties, it helps to prevent cancer and infectious diseases. Peels of citrus contain polymethoxylated flavones (PMFs), which have no negative side effects and can lower cholesterol more effectively than some prescription medications (Parle and Chaturvedi, 2012). There are numerous economically important species of citrus fruits. Few species, such as mandarins, lemons, limes, sweet oranges, and pomelo, are grown for commercial purposes in India. In India, 14.1 lakh metric tons of citrus are produced on an estimated 10.91 lakh hectares. In Maharashtra, citrus grows on 2.10 lakh hectares and yields 18.76 MT. In the Vidarbha region of Maharashtra, Nagpur mandarin is grown on 1.48 lakh hectares and yields 13.56 lakh tons annually (Anonymous, 2020-21). Mandarin (Nagpur Santra) is primarily grown in Satpura Hills in Central India's Vidarbha region (Maharashtra). One of the best mandarins in the world is from Nagpur.

At various stages of development, citrus trees are prone to a variety of viral, bacterial, and fungal diseases, reducing production and productivity. Fungi are known to be the most common citrus pathogen in the globe, causing yield losses of up to 20% (Lima *et al.*, 2011). A multitude of environmental factors on the Indian subcontinent encourage the growth as well as development of *Colletotrichum* spp. to infect various hosts. Among the members of the genus Colletotrichum, the most commonly reported plant pathogens in India are *Colletotrichum gloeosporioides*. It is believed to impact many hosts and cause specific symptoms (Gautam, 2014). Twig blight, root rot, dry rot, stem end rots, brown rot, melanose, anthracnose, pre-and post-harvest rot, citrus scab, and greasy spot are the most common fungal diseases of citrus. One of the most harmful citrus diseases in the world is called twig blight, which is brought on by the fungus *C. gloeosporioides* (Timmer *et al.,* 2000). *C. gloeosporioides* causes pre- and post-harvest disease of tropical fruit crops, causing plant damage and resulting in low fruit yield and quality (Prapassorn Bussaman *et al.,* 2012). Fungal infection causes a 10% to 20% citrus fruit drop (Randhawa and Singh, 1967; Tayade and Ingle, 1997). According to Singh (1971), citrus losses in Uttar Pradesh ranged from 10 to 15% due to *C. gloeosporioides*-induced wither tip and fruit rot.

An enormous number of synthetic pesticides are used worldwide to protect agricultural crops. Chemical fungicides used to control plant pathogenic fungi have the potential to increase crop yields while ensuring market quality and crop production stability. However, the exponential rise in fungicide use has resulted in the emergence of disease strains that are resistant to them as well as an overabundance of fungicide residues in the food chain. Alternatives to synthetic fungicides are required for better fungal disease management. Plant-derived chemicals are one of many options (Staub, 1991). So, there is a global effort to find better substitutes for these synthetic chemicals that do not have toxicological impacts on the ecology.

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Disease control and natural pest, whether directly or indirectly through the use of usual plant products comprising essential oils, is very promising (Regnault-Roger, 1997; Hammer *et al.,* 1999 Isman, 2000; Mohammad Maaz Akhtar *et al.,* 2014; Nasrin Rahman *et al.*, 2021). Complex volatile molecules termed essential oils are created in many plant parts and have been shown to perform a number of functions in plants, including resistance to pests and diseases (Goubran and Holmes, 1993).

Each essential oil is a complex blend of terpenes, including diterpenes, monoterpenes, terpenoids, and sesquiterpenes, as well as their oxygenated derivatives (phenols, alcohols, oxides, ketones, esters, and aldehydes), as well as phenolic and phenylpropanoid compounds derived from the acetate-mevalonic acid and shikimic acid pathways (Sil *et al.,* 2020). These oils are well-known to have antifungal properties against both human and plant diseases. Antifungal essential oils inhibit hyphal growth and cause fungi to lyse and evacuate their cytoplasm (Wijesekara *et al.,* 1997).

The antimicrobial potential of essential oils against the pathogen that causes citrus twig blight (*C. gloeosporioides*) in the Vidarbha area of Maharashtra has not yet been studied, despite research on the antifungal activity of many essential oils against pathogens. In the current study, essential oils from various plant origins had been examined at various concentrations for their capability to restrict the growth and spread of foliar plant diseases. This was done in light of the significance of essential oils in plant pathogen control.

2. Materials and Methods

The current research was conducted at the AICRP on Fruits Scheme Laboratory and the Department of Plant Pathology, Post Graduate Institute, Dr. PDKV, Akola, in 2021-22.

2.1 Collection of disease sample and isolation

To isolate the incitant, a twig of Nagpur mandarin exhibiting usual symptoms of twig blight disease was gathered from the field of AICRP on Fruits Scheme and subjected to a standard tissue isolation method. The culture was maintained on PDA (Potato Dextrose Agar) medium slants by sub-culturing as necessary at regular intervals after being purified using the hyphal tip technique. The detection of fungal pathogens involved in causing respective infected samples was resolved by conducting studies on their morphological and cultural features. Fungal spores or mycelium from the laboratory-grown cultures were examined under a high-power (40X) microscope to validate the identity of the fungus. *C. gloeosporioides* was recognised by its hyaline and septate mycelium, conidia with rounded ends and an oil globule in the centre and the presence of acervuli with erect setae. Pathogenicity tests in pot culture were performed in the glass house to prove Koch's postulates. Artificially infected leaves of Nagpur mandarin seedlings were used for re-isolation of the test pathogens. To validate their identification and pathogenicity, the acquired culture was tested for growth with the original culture collected from naturally diseased specimens.

2.2 Evaluation of plant essential oils by poisoned food technique

Nine commercially available plant essential oils, *viz*., neem (*Azadirachta indica*); eucalyptus (*Eucalyptus globules*); palmarosa (*Cymbopogan martini*); peppermint (*Mentha piperita*); olive oil (*Olea europaea*); thyme (*Thymus vulgaris*); lemongrass (*Cymbopogon winteriana*); citronella (*Cymbopogan nardus*) and garlic (*Allium sativum*) efficacy tested *in vitro* against *Colletotrichum gloeosporioides* at three distinct concentrations (0.1, 0.5 and 1%) with the Poisoned Food Technique (Nene and Thapliyal, 1993).

Prior to conducting the experiment, the test pathogen had been cultured in Petri plates for 10 days on a PDA medium. PDA was prepared and distributed in an amount of 100 ml in a 250 ml Erlenmeyer flask and autoclaved at 1.05 kg/cm^2 for 15 min. Using Tween 80 (0.05%) as an emulsion agent, the pure-grade essential oil was individually diluted at concentrations of 0.1, 0.5 and 1%. They were then added to autoclaved potato dextrose agar right before being poured onto Petri dishes. To ensure an even and consistent distribution of the essential oil, the flask containing the poisoned medium was thoroughly shaken. Each of the sterile Petri plates was filled with 20 ml of a poisonous solution, which was then allowed to solidify. These Petri plates were immunized by *C. gloeosporioides* culture. With the aid of a sterilized cork borer, a 6 mm disc of test pathogen coated with germinated active fungal growth was positioned in the middle of the medium. For this, ten days old culture of test pathogens in Petri dishes on a sterilized PDA medium was used. Three replications for test pathogen and control, specifically without the addition of any essential oil, were kept. Petri plates were incubated at a temperature of 25 ± 2 ^{0}C. The whole procedure was carried out under aseptic conditions. The fungal colony's linear diameter (2 pairs, each pair at right angles to the other) was measured daily until the mycelium in control completely filled the Petri plate. Experiment had been carried out in CRD (Completely Randomized Design) with 3 replications. Per cent inhibition of the test pathogen had been evaluated by applying the formula given by Ogbebor *et al.* (2007).

2.3 Evaluation of plant essential oils by volatile phase effect

Using volatile phase effects (Soylu *et al.,* 2006), nine commercially available plant essential oils with 3 distinct concentrations (0.1, 0.5, and 1%) were tested against *C. gloeosporioides.*

Potato dextrose broth was prepared and distributed at the rate of 100 ml in a 250 ml conical flask, autoclaved at 1.05 kg/cm^2 for 15 min. Each sterilized Petri plate (90 mm diameter) poured 20 ml of sterilized medium, which was allowed to solidify. A 6 mm sporulating disc of test pathogen was placed in the centre of the medium using a sterilized cork borer. A sterilized cork borer was used to place a 6 mm sporulating disc of the test pathogen in the centre of the medium. A ten days old culture of test pathogens in Petri dishes on sterilized PDA medium was used for this. On the inner surface of the inverted lid of Petri dishes, sterile filter papers (Whatman No. 1) were coated with various concentrations (0.1, 0.5, and 1%) of essential oils. Three replicates were retained for the test pathogens and the control, *i.e.*, where no essential oil has been added to the sterile filter paper. Petri dishes were incubated at 25 ± 2^{o}C temperature.

The whole procedure was carried out under aseptic conditions. At 24 h intervals, observations on radial mycelial growth and percent

inhibition of the test fungus by volatile phase effect were made and continued until the growth of the test pathogen in the untreated control plate was completely covered. Experiment was conducted in CRD with 3 replications. The method provided by Ogbebor *et al.* (2007) had been used to calculate the percent inhibition of the test pathogen.

2.4 Statistical analysis

Data for each trait were statistically tested using variance for different treatments. Following the process explained by Gomez and Gomez (1984), Fisher's protected CD (Critical Difference) test had been utilized to suggest a distinction between treatments at probability levels of p= 0.01.

3. Results

3.1 Sample collection and isolation of pathogen

Drying of twigs downwards (Figure 1) and covering numerous black fruiting bodies of the fungus on dead twigs that appear silvery grey in colour were collected from the Nagpur mandarin orchard of AICRP on Fruits, Dr. PDKV, Akola and brought in the laboratory to identify the cause and subjected to standard tissue isolation method. Using the tissue isolation method on a PDA medium, the pathogen was isolated from Nagpur mandarin diseased plant. *C. gloeosporioides* were recovered from tissue isolation methods in collected specimens. The isolated fungus was brought in pure culture using the hyphal tip method, and their cultural and microscopic characteristics were observed.

Figure 1: Twig blight of Nagpur mandarin.

3.2 Identifying isolated pathogen

The fungus, *C. gloeosporioides* was found in the majority of mandarin twig blight bits. The colony of isolates was creamy white to dark/light grey margin (Figure 2). During sporulation, a grey-black colon appeared in the colony's center. Isolate mycelium was initially hyaline, creamy white to light grey, later turned to light grey to black at centre with red salmon circular and creamy white with dark grey margins. Mycelial distinct features were initially thin, filamentous, and profusely branched but later became thick in compact colonies with entire thick margins.

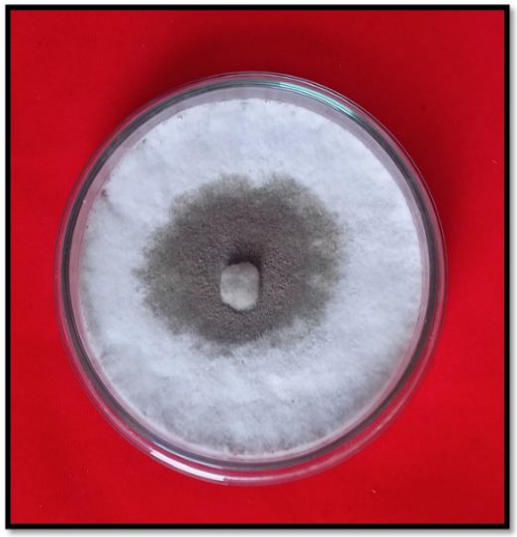

Figure 2: Pure culture of *C. gloeosporioides*.

In general, conidia were hyaline, one-celled, ovoid to oblong shape, with oil globules in the centre (Figure 3). The size of the isolate's conidia was measured at 10.40 × 3.5 µm. Acervuli were abundantly formed in the culture, and their shape ranged from elliptically globose to irregular. The setae were long, brown and septate.

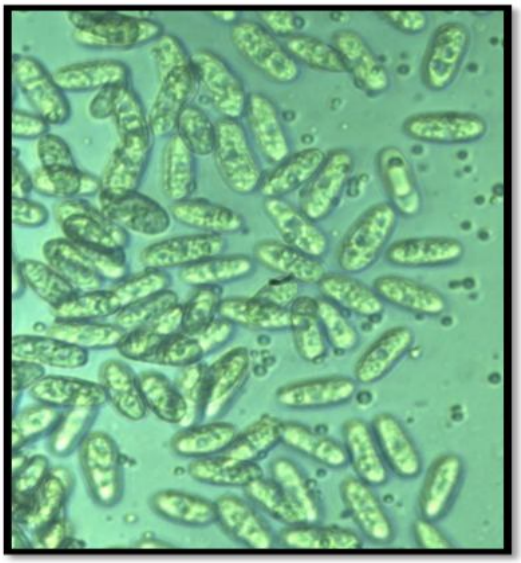

Figure 3: Microscopic view of *C. gloeosporioides*.

3.3 Evaluation of plant essential oils by poison food technique

The efficiency of nine plant essential oils at various concentrations for mycelial growth of *C. gloeosporioides* was tested *in vitro* using the Poison Food Technique.

3.3.1 Mycelial growth

The findings shown in Table 1 showed that all of the essential oils tested were significantly superior to the control in inhibiting *C. gloeosporioides* pathogen growth over the untreated control (90.00 mm) and that the concentrations of the essential oils tested decreased with increasing concentrations.

Table 1: Effect of plant essential oils on *C. gloeosporioides* radial mycelial growth using poisoned food technique (10 DAI)

S. No.	Essential oil	Concn. (%) and mycelial growth in mm			% Growth inhibition		
		0.1	0.5	1	0.1	0.5	1
1	Neem	61.38	50.16	41.44	31.80	44.26	53.95
2	Eucalyptus	33.25	21.79	14.42	63.05	75.78	83.87
3	Palmarosa	35.72	23.72	0.00	60.31	73.64	100.0
4	Peppermint	53.46	47.48	40.70	40.6	47.24	54.77
5	Olive	45.74	42.41	37.69	49.17	52.87	58.12
6	Thyme	83.36	72.96	64.50	7.37	18.93	28.33
7	Lemongrass	0.00	0.00	0.00	100.0	100.0	100.0
8	Citronella	0.00	0.00	0.00	100.0	100	100.0
9	Garlic	0.00	0.00	0.00	100.0	100.0	100.0
10	Control	90.0	90.0	90.0			
	SE (m) ±	0.46	0.35	0.4			
	CD (p=0.01%)	2.14	1.64	1.89			

DAI: Days after inoculation.

Essential oils inhibited the growth of *C. gloeosporioides* at 0.1% concentration, with radial mycelial growth ranging from 00.00 mm (citronella, lemongrass, and garlic oil) to 83.36 mm (thyme oil) compared to 90.00 mm in the untreated control. However, citronella oil, lemongrass oil and garlic oil were found to be highly efficient in inhibiting the pathogen with the least mycelial growth (00.00 mm). Following this, eucalyptus oil (33.25 mm), palmarosa oil (35.72 mm), olive oil (45.74 mm), peppermint oil (53.46 mm) and neem oil (61.38 mm). With a maximum mycelial growth of 83.36 mm, thyme oil was observed to be the least efficient of all essential oils (Figure 4).

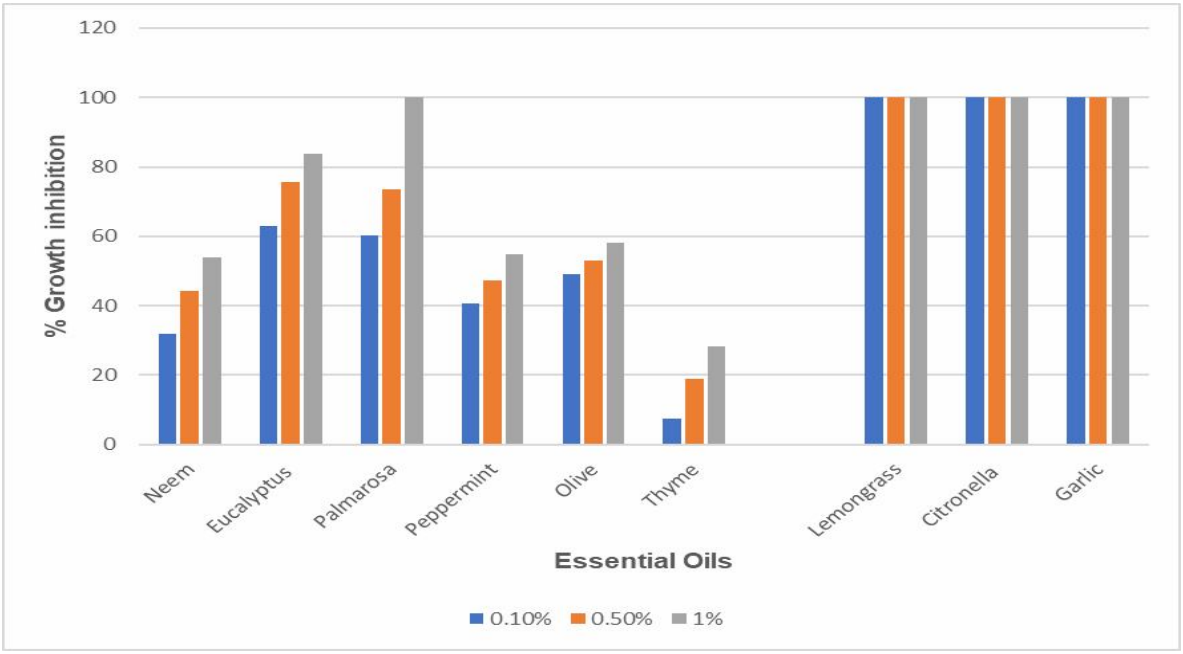

Figure 4: Effect of essential oils on radial mycelial growth of *C. gloeosporioides* by poisoned food technique (10 DAI).

At 0.5% concentration, among the effective essential oils, citronella oil, lemongrass oil, and garlic oil (0.00 mm) had no growth of *C. gloeosporioides*, proving to be significantly superior. The next in order of merit was eucalyptus oil (21.79 mm), followed by palmarosa oil (23.72 mm), and the rest of the essential oils had a comparatively lower inhibitory effect.

At 1% concentration, essential oils inhibited the growth of *C. gloeosporioides*, with radial mycelial growth ranging from 00.00 mm (citronella, lemongrass, palmarosa and garlic oil) to 64.50 mm (thyme oil) as against 90.00 mm in the untreated control. Citronella oil, lemongrass oil, palmarosa oil and garlic oil were observed to be the most efficient at inhibiting the pathogen with the least amount of mycelial growth (00.00 mm). Eucalyptus oil was the next best treatment, with 14.42 mm mycelial growth. Thyme oil had been observed to be less efficient when compared to other essential oils, with a maximum mycelial growth of 64.50 mm. Essential oil antifungal activities were dose-dependent, with higher doses exhibiting stronger antifungal activities.

3.3.2 Mycelial growth inhibition

C. gloeosporioides mycelial growth inhibition ranged from 7.37% (thyme oil) to 100% (citronella oil, garlic oil, and lemongrass oil,) at 0.1% concentration. Citronella oil, lemongrass oil, and garlic oil inhibited 100% of pathogen mycelial growth, followed by eucalyptus oil (63.05%). Palmarosa oil (60.31%), olive oil (49.17%), peppermint oil (40.6%) and neem oil (31.8%) were the next best treatments. Among all essential oils, thyme oil was found to be the least effective, with only 7.37 per cent mycelial inhibition of pathogens observed.

Citronella oil, lemongrass oil and garlic oil inhibited 100% mycelial growth of pathogens at 0.5% concentration, followed by eucalyptus oil (75.78%), palmarosa oil (73.64%), olive oil (52.87%), peppermint oil (47.24%) and neem oil (44.26%) were the next best essential oils. Thyme oil was observed to be the least efficient of all essential oils, with only 18.93% mycelial inhibition of pathogens recorded.

At 1% concentration, citronella oil, palmarosa oil, lemongrass oil and garlic oil inhibited 100% mycelial growth of *C. gloeosporioides*. Next best was eucalyptus oil (83.87%), followed by olive oil (58.12%), peppermint oil (54.77%) and neem oil (53.95%). Thyme oil was observed to be the least effective amongst all essential oils, with only 28.33 per cent mycelial inhibition of pathogens observed.

Citronella oil, palmarosa oil, lemongrass oil and garlic oil inhibited growth the most, followed by eucalyptus oil. This experiment demonstrates that essential oil, citronella oil, palmarosa oil, lemongrass oil and garlic oil may contain a strong toxic principle that directly affects the growth of *C. gloeosporioides*, the causal agent of twig blight of Nagpur mandarin.

3.4 Evaluation of plant essential oils by volatile phase effect

3.4.1 Mycelial growth

With exception of thyme oil, Table 2 demonstrates that all of the tested essential oils showed a wide range of *C. gloeosporioides* radial mycelial development above the untreated control (90.00 mm), which was observed to be diminished with greater doses of the tested essential oils.

The growth of *C. gloeosporioides* growth was inhibited by essential oil at a concentration of 0.1%, with radial mycelial growth ranging from 00.00 mm - 90.00 mm. Citronella, lemongrass, palmarosa and garlic oil were observed to be quite efficient in inhibiting pathogens with the least mycelial growth (00.00 mm), followed by neem oil (31.32 mm). These treatments were followed by olive oil (32.62 mm), peppermint oil (65.49 mm) and eucalyptus oil (71.57 mm). Thyme oil was relatively ineffective in the volatile phase, with a maximum mycelium growth of 90.00 mm.

At 0.5% concentration, the same trend was observed, with citronella oil, lemongrass oil, palmarosa oil and garlic oil being found to be the most effective at inhibiting the pathogen with the least amount of mycelial growth.

Table 2: Effect of essential oils on *C. gloeosporioides* radial mycelial growth of using volatile phase effect (10 DAI)

S. No.	Essential oil	Concn. (%) & mycelial growth in mm			% Growth inhibition		
		0.1	0.5	1	0.1	0.5	1
1	Neem	31.32	29.51	27.53	65.2	67.21	69.41
2	Eucalyptus	71.57	69.89	61.27	20.47	22.34	31.92
3	Palmarosa	0.00	0.00	0.00	100	100	100
4	Peppermint	65.49	62.67	35.72	27.23	30.36	60.31
5	Olive	32.62	30.38	15.85	63.65	66.24	82.38
6	Thyme	90.0	90.0	90.0	0.00	0.00	0.00
7	Lemongrass	0.00	0.00	0.00	100	100	100
8	Citronella	0.00	0.00	0.00	100	100	100
9	Garlic	0.00	0.00	0.00	100	100	100
10	Control	90.0	90.0	90.0			
	SE (m) ±	0.34	0.38	0.37			
	CD (p=0.01%)	1.604	1.766	1.745			

DAI: Days after inoculation.

When used at 1% concentration, essential oils showed radial mycelial development ranging from 0.00 mm (citronella oil, lemongrass oil, palmarosa oil and garlic oil) to 90 mm (thyme oil), as opposed to 90.00 mm in the untreated control. Citronella oil, lemongrass oil, palmarosa oil and garlic oil were observed to be the most efficient in inhibiting the pathogen, with the least mycelial growth (00.00 mm), followed by olive oil (15.85 mm). These treatments were followed by neem oil (27.53 mm), peppermint oil (35.72 mm) and eucalyptus oil (61.27 mm). Thyme oil was found to be the least potent of all essential oils, with a maximum mycelial growth of 90.0 mm.

With a maximum mycelial growth of 90.0 mm, thyme oil was observed to be the least efficient of all essential oils.

3.4.2 Mycelial growth inhibition

Table 2 shows that all nine essential oils tested (at 0.1%, 0.5%, and 1% concentrations) significantly inhibited *C. gloeosporioides* mycelial growth compared to the untreated control. As per the findings, the effects of three concentrations differed significantly at $p<0.01$. The results revealed that all concentrations of citronella oil, lemongrass oil, palmarosa oil and garlic oil entirely inhibited mycelial growth of the tested pathogen. The next best essential oil was neem oil, which inhibited 65.20, 67.21, and 69.41% at 0.1, 0.5, and 1% concentrations, respectively, followed by olive oil. At 1% concentrations, the next best percent inhibition (82.38%) was obtained in olive oil. However, thyme oil exhibited no inhibition.

4. Discussion

The characteristics of *C. gloeosporioides* matched with those reported by Carrington *et al.* (2002), Hyde *et al.* (2009) and Weir *et al.* (2012). Carrington *et al.* (2002) investigated the colony characteristics and conidial morphology of *C. gloeosporioides*, a citrus pathogen that causes premature fruit drop. They reported that the majority of isolates on the PDA had a dense and white-grayish colony with above ground mycelium and dark brown to black conidial masses. Conidia varied in size from 7.5 to 17.5 × 2.5 to 3.75 µm, with hyaline, straight and rounded conidia at both ends. While researching the citrus wither twigs disease, Benyahia *et al.* (2003) observed white mycelial growth of *C. gloeosporioides* on PDA. The isolate exhibited similar morphological characteristics in the current investigation, confirming previous reports published by different workers.

On the basis of morphological features and published literature, the fungal identification was established. Also, it is clear that *C. gloeosporioides* caused twig blight in Nagpur mandarin. Under greenhouse conditions, Goes and Kimati (1997) investigated the pathogenicity of *C. gloeosporioides* and *C. acutanum* on citrus and reported the typical symptoms in the form of orange necrotic lesions on a twig, leaves and in post-bloom fruit drops of citrus. Similar symptoms were noticed in the present investigation of the pathogenicity test.

The essential oils used in this research are antifungal and commercially available. To assess the antifungal activity of test oils against twig blight incitant, an assay was performed on poison food agar medium. This assay technique gives qualitative data on the efficiency of the test compounds. We found significant inhibition in the poison food assay, indicating that the essential oils have antifungal activity.

Previously, researchers reported on the effects of various essential oils on *C. gloeosporioides*. According to Alemayehu Chala *et al.* (2014), essential oils have inhibitory effects on the mycelial growth of *C. gloeosporioides* isolates *in vitro*. They found that oils derived from white cumin and palmarosa were better in terms of antifungal activity, inhibiting fungal growth even at low concentrations. Lemon grass oil also showed very strong antifungal activity against *C. gloeosporioides*, as it completely inhibited fungal growth at high concentrations. The same oil at 0.25% reduced mycelia growth by 80.20% when compared to the control. Jeong *et al.* (2009) investigated the impact of lemongrass oil on the linear growth of *C. gloeosporioides* at six concentrations (0.2%,0.1%,0.05%, 0.125%, 0.0625% and 0%). Lemongrass oil had the inhibitoriest effect on *C. gloeosporioides* fungi. Consistent with this, Samithri *et al.* (2020) found that cardamom and citronella oils greatly inhibited mycelial growth of Colletotrichum species, but lemon, orange, and mustard oil had no such effect.

In many cases, essential oil inhibitory effects increase with increasing concentration, confirming earlier findings by Palhano *et al.* (2004), who reported increased inhibitory effects of citralon on *C. gloeosporioides* spore germination at greater concentrations. Similarly, Jeong *et al.* (2009) reported that *C. gloeosporioides*, a plant pathogenic fungus, is controlled by essential oil and the addition of 1% essential oil entirely inhibited fungus growth compared to 0.5% concentrations even after 5 days of culture.

The antimicrobial potential of essential oils, particularly citronella, palmarosa, lemongrass, and garlic oil, may be because of their capacity to damage enzymatic cell systems, including those involved in energy production and synthesis of structural compounds (Lagrouh *et al.,* 2017). The inhibitory effects of these oils have also been correlated with high levels of monoterpenes, sesquiterpenes, phenols, flavonoids, quinons, alkaloids, tannins and steroids (Paranagama *et al.,* 2003). The concentrations of essential oils varied in their ability to inhibit mycelial growth of *C. gloeosporioides*. The chemical makeup of the oil, oil concentrations, variability of isolate, and experimental circumstances may influence the efficiency of the essential oil.

Results indicated that essential oils had a greater inhibitory effect on radial mycelial growth and percent growth inhibition as oil concentrations increased. On the other hand, when palmarosa oil was tested by volatile phase, all three concatenations showed 100% growth inhibition, which was not seen with the poison food method. As anticipated, the major antibacterial component of essential oil, monoterpene, was more potent than crude oil (Palhano *et al.*, 2004).

Antifungal volatile phase effects of numerous essential oils against *C. gloeosporioides* were reported earlier by researchers. The result of the current findings is nearly identical to Ali *et al.* (2015), who observed that lemongrass oil vapour significantly inhibited *C. gloeosporioides* mycelial growth. Furthermore, Shaon *et al.* (2016) reported that volatile essential oils such as citronella, eucalyptus, neem and karanj had excellent broad-spectrum antifungal activity against ten selected plant fungal pathogens, including *C. gloeosporioides.*

The antimicrobial activity of the volatile phase effect of essential oil was observed in the current study. This could be due to the volatile nature of the aroma component; the stronger the aroma, the

higher the concentration of vapour in the surrounding air space. Many essential oils contain volatile active ingredients that prevent pathogen growth. Essential oils inhibit fungal growth by preventing hyphal growth and sporulation, causing significant damage to the cellular organelle, cell membrane and cell wall, interfering with nutrient uptake and metabolism, disrupting plasma membrane and interfering with respiratory enzymatic reactions of the mitochondrial membrane (Wijesekara *et al.,* 1997; Riolo *et al.,* 2021; Itzaz Aslam *et al.,*2017).

5. Conclusion

Citronella oil, lemongrass oil, palmarosa oil, and garlic oil all showed higher levels of antifungal activity against *C. gloeosporioides* after proper laboratory testing, indicating that these essential oils could be utilized as alternatives to synthetic chemicals for integrated management of diseases caused by *C. gloeosporioides* in citrus crops. Further investigation is therefore required to create practical control methods for the *C. gloeosporioides*-induced twig blight disease.

Acknowledgments

Authors are immensely thankful to the AICRP on Fruits scheme and Department of Plant Pathology, Dr. PDKV, Akola for their cooperation and facilities in carrying out this research work.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **V. S. Pol, Y. V. Ingle and K. A. Lengare (2023). Antifungal effects of plant essential oils against Colletotrichum gloeosporioides, the fungus associated with citrus twig blight. Ann. Phytomed., 12(1):538-545. http://dx.doi.org/10.54085/ap.2023.12.1.12.**